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Influence of Nitrogen Dioxide on Xenobiotic Metabolism in Animals

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INFLUENCE OF NITROGEN DIOXIDE ON XENOBIOTIC METABOLISM IN ANIMALS*

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ABSTRACT

Potential extrapulmonary effects of nitrogen dioxide (NO_2) on hepatic xenobiotic metabolism were examined. Initial studies were conducted using pentobarbital (PEN)-induced sleeping time (S.T.) in mice as an indicator of integrated mechanisms of xenobiotic clearance. A 3 hr. exposure to concentrations as low as 0.47 mg NO_2/m^3 (0.25 ppm) caused a significant increase in PEN-induced S.T. in female mice. When exposures were repeated for several days, the magnitude of the effect diminished. Investigation of sex sensitivity indicated that female mice were affected by acute exposure (3 hr./day, 1 or 2 days), but males were not. It was also observed that a 4 day (3 hr./day) exposure to 1.88 mg NO_2/m^3 (1.0 ppm) induced tolerance to the effects of 9.4 mg NO_2/m^3 (5.0 ppm). Mechanisms for the increase in PEN-induced S.T. were sought by evaluating hepatic cytochrome P-450 levels and the activity of 3 hepatic mixed function oxidases (aniline hydroxylase, aminopyrine N-demethylase and p-nitroanisole O-demethylase) in female mice exposed to concentrations of NO_2 causing an increase in PEN-induced S.T. No significant effects were observed. A 3 hr. exposure to 9.4 mg NO_2/m^3 increased cytochrome P-450 levels in the lung, but not the liver, of rabbits.

INTRODUCTION

Ambient concentrations of ozone (O_3) [1,2] increased pentobarbital (PEN)-induced sleeping time (S.T.) in female mice, rats, and hamsters. Thus, it was hypothesized that another oxidant pollutant, nitrogen dioxide (NO_2), might have a similar effect. Since alterations in PEN-induced S.T. are commonly assumed to be representative of changes in the hepatic cytochrome P-450 (cyt. P-450) system, the investigation of NO_2 became interesting to us because extrapulmonary effects of NO_2 are rarely studied.

For the reasons noted above, the influence of NO_2 on PEN-induced S.T. was studied [3]. To determine potential target sites of NO_2 or its reactive products which might relate to changes in mixed function oxidases, selected hepatic enzymes were investigated [4]. The level of hepatic cyt. P-450 and

selected metabolic processes (O-demethylation, N-demethylation, and hydroxylation) were measured. Aryl hydrocarbon hydroxylase was not assayed since others [5,6] found no effect of NO_2 on this enzyme in the lungs of rabbits or rats. Because it was considered likely that the pulmonary cyt. P-450 system might also be affected, preliminary studies were conducted and described here.

METHODS

Animals and NO_2 exposures

Female CD-1 mice (20-25g) from Charles River Farms, Inc. (Wilmington, MA) and male rabbits (2 kg) from Dutchland (Denver, PA) were used. Mice were maintained on a bedding of white pine shavings and rabbits were maintained without bedding. Food and water were available ad libitum. A 12 hr photoperiod was maintained. Mice were used for all studies except for lung cyt. P-450 in which rabbits had to be used to obtain sufficient concentrations to measure levels in individual animals.

NO_2 exposures have been described [3]. Briefly, on any given day one group of animals was exposed to NO_2 and the other group received an identical exposure to clean air.

Pentobarbital-induced sleeping time

This technique has been described previously [1,2,3]. At 1:00 PM ± 15 min (immediately after NO_2 exposure) mice were placed in a temperature- (24 ± 1°C) and humidity-(46 ± 2% RH) controlled room to minimize the influence of circadian cycles and temperature. Mice were injected intramuscularly with 50 mg/kg of PEN (diluted Nembutal). The time to the loss of the righting reflex and the S.T. (the time between the loss and regaining of the righting reflex) were measured with stopwatches. Because the time to the loss of the righting reflex was not altered by NO_2, these data will not be presented or discussed.

Mixed function oxidases

The techniques used for mice have been described [4]. Immediately after exposure (1:00 PM ± 15 min), mice were killed by cervical dislocation. Standard techniques were used to measure cyt. P-450 levels. Enzyme assays were performed on the supernatant (16,000xg x 15 min at 4°C) of homogenates of individual mouse livers using slight modifications of established techniques. Each assay was made in duplicate. For p-nitroanisole O-demethylase activity, p-nitrophenol was measured; for aminopyrine N-demethylase activity, formaldehyde was measured; and for aniline hydroxylase, p-aminophenol was measured.

Lung cyt. P-450 levels were determined in rabbits using a standard method [7].

Statistical analyses

The data on PEN-induced S.T. in mice and rabbit lung cyt. P-450 levels were analyzed with a two-way analysis of variance with appropriate blocking factors. Hepatic enzymes were analyzed with a one-way analysis of variance. If required, Dunnett's test was used to determine the pattern of significant differences.

RESULTS

Effect of NO_2 on PEN-induced S.T.

To determine whether there was a sex susceptibility to the effect of NO_2, males and females were compared after a single or repeated 3 hr exposure to 9.4 mg NO_2/m^3 (5.0 ppm) or air [3] (Fig. 1). Females had a significant increase in PEN-induced S.T. after the first and second, but not the third, day of exposure. In contrast, males were only affected by the 3 days of repeated exposure. Because females were more sensitive to a single exposure, remaining studies were conducted with female mice.

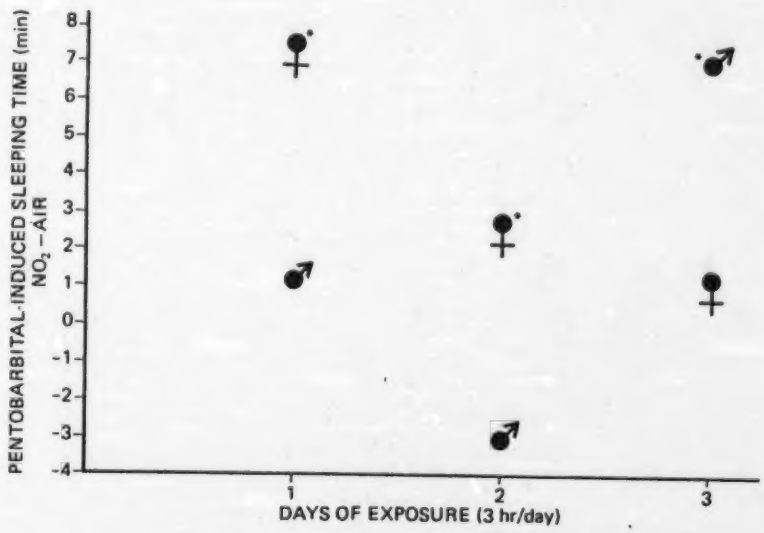

Fig. 1. Effect of exposure (3 hr./day) to 9.4 mg NO_2/m^3 (5.0 ppm) on PEN-induced S.T. in male (♂) and female (♀) mice. $^*p < 0.05$.

Concentration and time response studies were conducted with female mice exposed for 3 hr/day for up to 3 days [3] (Fig. 2). The lowest effective concentration tested was 0.47 mg NO_2/m^3 (0.25 ppm). Except for the exposure to 0.94 mg NO_2/m^3 (0.5 ppm), all effective concentrations caused a maximal increase in PEN-induced S.T. on the first day which dissipated thereafter.

Because tachyphylaxis appeared to occur, an additional experiment was performed in which female mice were exposed to 1.88 mg NO_2/m^3 (1.0 ppm) for 3

hr/day for 4 days and on the fifth day were exposed for 3 hr to 9.4 mg NO_2/m^3 (5.0 ppm) [3]. The PEN-induced S.T. of the pre-exposed NO_2 group (20.4 min. ± 0.7 S.E.; n=45) did not differ ($p > 0.1$) from the control air group (21.2 min. ± 0.6 S.E.; n=44). However, as depicted on Fig. 2, a single 3 hr exposure to 9.4 mg NO_2/m^3 (5.0 ppm) increased ($p < 0.05$) PEN-induced S.T. (28.9 min. ± 1.2 S.E.; n=59) when compared to the air control (21.4 min. ± 0.4 S.E.; n=59). Thus, the pre-exposure to the lower concentration rendered the mice tolerant to the higher exposure.

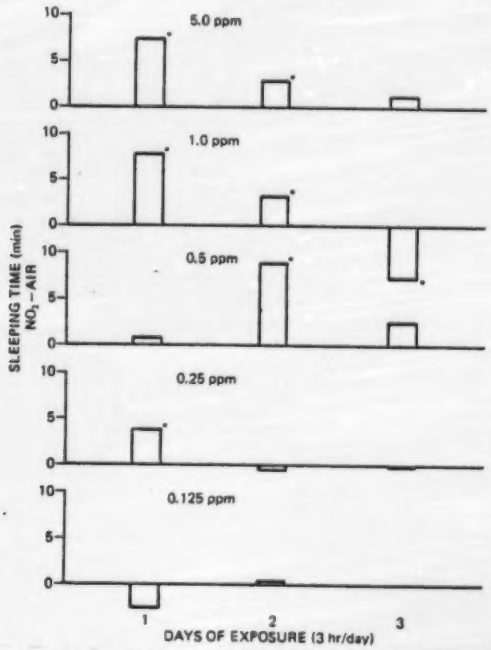

Fig. 2. Effect of NO_2 on PEN-induced S.T. in female mice. *$p < 0.05$.

Effect of NO_2 on hepatic mixed function oxidases

Female mice were exposed for 3 hr to 9.4 mg NO_2/m^3 (5.0 ppm) or air and hepatic cyt. P-450 concentrations were measured [4]. As shown in Table 1, no effects ($p > 0.1$) were observed.

TABLE 1

Effect of 5 ppm NO_2 on hepatic cytochrome P-450.

Exposure	Number of mice	nmol cyt. P-450/mg protein
Air	18	0.68 ± 0.1[a]
NO_2	18	0.69 ± 0.08

[a]All data are mean ± S.E.

The hepatic enzyme activities were also assayed after a 3 hr exposure to air or 9.4 mg NO_2/m^3 (5.0 ppm) [4]. NO_2 caused no effects ($p > 0.1$) as shown in Table 2.

TABLE 2

Effect of 5 ppm NO_2 x 3 hr on hepatic enzyme activities.

Enzyme	Exposure	nmol Product/mg Protein/min[a]
Animopyrine N-demethylase	AIR	0.0062 ± 0.0004
	NO_2	0.0054 ± 0.0003
p-Nitroanisole O-demethylase	AIR	1.13 ± 0.06
	NO_2	1.15 ± 0.10
Aniline hydroxylase	AIR	0.99 ± 0.07
	NO_2	0.95 ± 0.07

[a]All data are mean ± S.E., 15 mice/group. See methods for product measured and ref. 4 for details of technique used.

Effect of NO_2 on lung cyt. P450 levels

Since there were no effects of NO_2 on liver cyt. P-450 levels or enzyme activities, it was of interest to determine if xenobiotic metabolism of the lung would be more affected. Because lung concentrations of cyt. P-450 are considerably lower than liver levels, rabbits were used instead of mice to achieve measurable levels. To date, only a preliminary experiment has been completed. Rabbits were exposed for 3 hr to air or 9.4 mg NO_2/m^3 (5.0 ppm) and cyt. P-450 levels were determined immediately or 3, 5, or 7 days post-exposure. The post-exposure periods were examined since Goldstein et al. [8] observed that O_3 caused more of a change in lung cyt. P-450 at post-exposure times. NO_2 caused no change in hepatic cyt. P-450 levels ($p > 0.1$). However, the concentration of cyt. P-450 in the lung was increased (Fig. 3).

DISCUSSION

The major mechanism for the termination of S.T. induced by PEN is aliphatic hydroxylation by cyt. P450, as opposed to cyt. P-448, metabolism [9,10]. The liver appears to be the predominant site of PEN metabolism [11]. Therefore, it appears possible that NO_2 may have increased PEN-induced S.T. by altering hepatic PEN metabolism. The mechanisms responsible are not known, particularly since in the investigations reported here, NO_2 caused no effects on

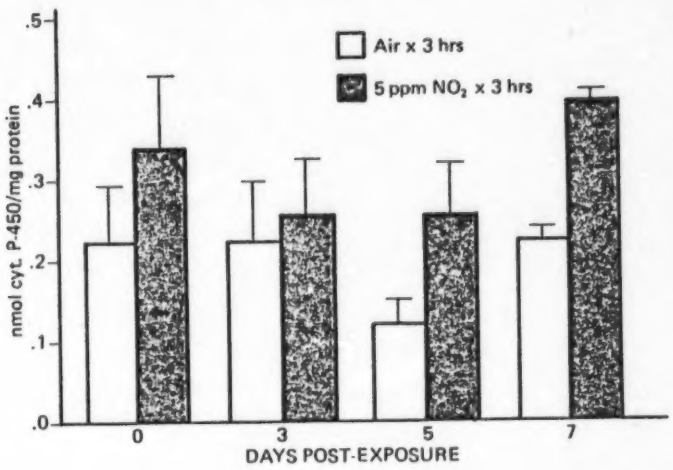

Fig. 3. Effect of a 3 hr exposure to 9.4 mg NO_2/m^3 (5 ppm) on hepatic cyt. P-450 levels. The experiment consisted of 2 replicates. The final number of animals per group was 3-4, except for day 7 for which 2 rabbits were used. The analysis of variance indicated that NO_2 caused an increase ($p < 0.05$) in cyt. P-450 levels.

hepatic cyt. P-450 levels or selected enzyme activities in vitro. In vivo measurements of mixed function oxidase activities are needed, however, before the potential involvement of these enzymes are ruled out. Such a study is now underway in which the effect of O_3 on lindane metabolism is being investigated.

It is probable that NO_2 itself, because of its reactivity, did not reach the liver. However, reactive products of NO_2 are likely to be formed in the lung [12], and fatty acid epoxides have been detected in the lungs of rats exposed to NO_2 [13]. Mechanisms of transport of epoxides or other potential reactive products to the liver would be speculative, but are possible since distribution studies with labeled NO_2 have shown that the label is transported to extrapulmonary sites almost immediately after inhalation [18]. Others [15] have shown that within a few days after the skin painting of mice with choles terol-α-epoxide, the epoxide could be found in several organs, including the liver.

In most cases, NO_2 and O_3 cause similar effects, the major difference being that usually higher levels of NO_2 (compared to O_3) are needed to cause the response [16]. The results of the concentration-response study with NO_2 therefore pose an interesting contrast to O_3. With O_3, as the concentration decreases, it takes an increasing number of days of exposure (3 hr/day) to cause an increase in PEN-induced S.T. [2]. For example, 1.96 mg O_3/m^3 (1.0

ppm) was the lowest concentration that caused an effect after a single 3 hr exposure. At 0.49 mg O_3/m^3 (0.25 ppm), 6 days (3 hr/day) were required to produce an effect, whereas a a single 3 hr exposure to 0.47 mg NO_2/m^3 (0.25 ppm) produced a significant increase in PEN-induced S.T. This is the only study we are aware of which showed that an acute exposure to NO_2 was more potent than O_3. Another difference observed is that pre-exposure to a lower level of NO_2 results in tolerance to a higher concentration of NO_2. No such tolerance is observed for PEN-induced S.T. with O_3 [1], although for other effects like edema [16,17], both gases produce tolerance.

The reason for the sex difference in the effect of NO_2 on PEN-induced S.T. is not known. Mice, like most other species except the rat, have only minor sex differences in drug metabolism [18]. Female mice were also more sensitive to O_3 [2], but unlike NO_2, repeated exposure to O_3 did not increase PEN-induced S.T. in male mice.

Studies with hepatic mixed function oxidase inducers have also indicated differences between NO_2 [19] and O_3 [20]. Phenobarbital, which causes a general induction, caused a decreased survival time of rats in O_3, but an increased survival time in NO_2. 3-Methylcholanthrene, which predominately induces cyt. P-448, increased survival time in NO_2, but not in O_3. Both inducers decrease lipid peroxidation in rat liver following exposure to carbon tetrachloride [21]. Since lipid peroxidation is a major factor in NO_2 and O_3 toxicity [12] and lipid peroxides can destroy cyt. P-450 systems [22,23], the differences between the NO_2 and O_3 responses with these inducers are not explained easily. Confounding this issue is that the lethality of the gases was most probably caused by pulmonary edema, and thus cyt. P-450 system induction in the lung may have had a greater role than induction in the liver which is the primary site of PEN metabolism. Nevertheless, these mortality studies do indicate the potential involvement of cyt. P-450 systems in oxidant toxicity. The preliminary NO_2 studies in rabbits show that NO_2 increases the level of cyt. P-450 in the lung, but not the liver. It was unusual that there was no statistically significant interaction with the post-exposure examination times (i.e. there was no recovery over the times studied). This is in contrast to O_3 [8] which decreases cyt. P-450 in the lung without affecting liver levels. Previous work [5] had shown that very high levels of NO_2 (94 mg/m^3, 50 ppm) caused no change in lung benzo(a)pyrene hydroxylase activity in rabbits, although O_3 (1.47 mg/m^3, 0.75 ppm) decreased this activity. Hopefully further research now underway will clarify this issue.

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